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Original Research Article

Identification markers based on fatty acid composition to differentiate between roasted *Arabica* and *Canephora* (*Robusta*) coffee varieties in mixtures

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ABSTRACT

Commercial coffee is available as a mixture of two varieties of coffee beans, namely *Arabica*, which is more expensive, and *Canephora* (*Robusta*), less expensive. To assess the correspondence between the composition indicated on the label and the real composition of commercially available coffee, it would be desirable to be able to differentiate between the two varieties. This would also help to avoid any possible commercial frauds. This work identifies parameters based on the fatty acid composition to differentiate between *Arabica* and *Canephora* coffee in a mixture. Total monounsaturated fatty acids (ΣMUFA), linolenic acid (*cis*18:3*n*–3) concentration, the 18:0/*cis*18:1*n*–9 ratio, and the ΣMUFA/ΣSFA ratio could be used to determine the relative amounts of *Arabica* and *Canephora* in a coffee blend.

1. Introduction

Coffee, with a market share of about $US 15 billion (Mussatto et al., 2011) is the second most marketed commodity worldwide after oil. The most cultivated varieties are Coffea *Arabica* (*Arabica*) and Coffea *Canephora* (*Robusta*). *Arabica* coffee is of higher quality *than Canephora* coffee; it has a lower caffeine content and the drink obtained from the *Arabica* bean is sweeter to the taste, has an aromatic fragrance and a rounded flavour (Carrera et al., 1988).

Coffee is commonly marketed as a mixture of the two varieties blended in different amounts but the prices of the two pure varieties are different. The variety *Arabica* is more expensive and consequently the price of commercially available mixtures is mainly connected to the percentage content of *Arabica*. This is mostly due to the necessity of controlling the market price, but also to offer the end-user a product adequately structured from the sensory point of view. There is a possibility that commercial coffee could contain less *Arabica* than the composition indicated on the label, constituting *in se* a commercial fraud (Downey and Boussion, 1996). As a consequence, a method to differentiate between coffee *Arabica* and *Robusta* content in a mixture would be highly advisable.

Many studies in the literature report methods to identify indicators that can distinguish between *Arabica* and *Canephora* in a mixture. Carrera et al. (1988) proposed sitostanol and Δ^5avenasterol as possible discriminating indicators. The lipid component of coffee has also been proposed as a tool to differentiate *Arabica* and *Robusta* (Valdenebro et al., 1999). Other possible markers have been proposed in recent years, *e.g.*, caffeine and free amino acids content (Martin et al., 2001), triglycerides trilinolein (LLL) and 1,3 dioleyl-2 linoleyl glycerol (OLO) content (González et al., 2001), and 16-*O*-methylcafestol content (Campanha et al., 2010). The abovementioned authors outlined that the concentration of the studied marker molecules was highly dependent on the degree of roasting of the raw material and, consequently, obtained results were affected by a large variability.

The fatty acid composition of *Arabica* and *Robusta* roasted coffee has been extensively studied (Folstar, 1985; Ratnayake et al., 1993; Lercker et al., 1996a,b). Martin et al. (2001) suggested the amounts of myristic, oleic, linoleic and linolenic acids as possible

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discriminators of *Arabica* and *Canephora* varieties, stating also that the fatty acid composition (%, w/w) was not affected by the thermal treatment adopted in the coffee roasting process (Muratore et al., 1998; Karl Speer and Kölling-Speer, 2006). Nevertheless, the reported experiments analysed only monovarietal samples of *Arabica* and *Canephora* from different geographic areas and not mixtures of the two (Martin et al., 1998). The availability of a reliable analytical method based on fatty acid composition as a tool to assess the relative amounts of *Arabica* and *Robusta* in a mixture would be highly desirable, and could represent a useful tool to prevent possible fraud in commercial coffee blends.

2. Materials and methods

2.1. Sampling

Bags of 5 kg of roasted coffee beans of *Arabica* (A) and *Robusta* (R) cultivars collected during the harvest of the year 2010/2011 from a reliable and traceable source were purchased from a major Italian coffee producer which guaranteed the origin and provenance. The coffee bags were sealed and stored under modified atmosphere to prevent oxidation or other possible compositional modification. Table 1 shows the individual varieties of *Arabica* and *Robusta* analysed and their geographical origin. The same amount of each variety was sampled and then blended to form the samples labelled as MR and MA, each having a 1 kg final weight. The samples MR and MA were appropriately combined to obtain 8 mixtures of 100 g each containing different percentages of *Arabica* coffee as detailed in Table 2. These samples were analysed and the results used to build calibration curves. Moreover, 13 commercial blends of coffee, whose composition was indicated on the label, were obtained from local market. The coffee was stored in properly sealed packs of 250 g. Finally, 6 additional mixtures of known coffee *Arabica* composition (87%, 73%, 65%, 50%, 30% and 20%, w/w), as reported in Table 3, were prepared in the laboratory.

2.2. Lipid extraction from coffee

For the lipid extraction from coffee, 200 g of coffee grains of each analysed mixture were ground in an electric grinder (Moulinex, SEB Group, Selongey, France) to a particle diameter of 20–30 μm. The lipid fraction of the coffee was extracted from the roasted ground beans (powdered) using the solid–liquid extraction method and a Soxhlet extractor SER 148 (Scientific Velp, Usmate, Monza Brianza, Italy). AOAC (Association of Official Analytical Chemists) official method of analysis (AOAC, 1965. Method 14.029) was used.

Table 1
Coffee Arabica (Arabica) and coffee Canephora (Robusta) varieties and their mixtures and their geographic origin. Identification code names for used roasted coffees are given.

Coffee varieties	Origin	Identification code
Arabica	Panama	A1
Arabica	Colombia	A2
Arabica	Brazil	A3
Arabica	Ethiopia	A4
Arabica	Guatemala	A5
Arabica	Brazil	A6
Robusta	Uganda	R1
Robusta	Congo	R2
Robusta	Congo	R3
Robusta	Zaire	R4
Robusta	Zaire	R5
Mixture (R1, R2, R3, R4, R5)		MR
Mixture (A1, A2, A3 T, A4, A5, A6)		MA

Table 2
Mixtures of coffee used to obtain the calibration line; MR and MA indicate single *Canephora* and *Arabica* coffee varieties, respectively.

Mixtures MR/MA	% (w/w) coffee *Arabica*
M1	10
M2	20
M3	30
M4	40
M5	50
M6	60
M7	70
M8	80
M9	90

2.3. Fatty acids analysis

The fatty acids composition was obtained by gas chromatography (GC) after derivatisation to fatty acid methyl esters (FAME) with 2 N KOH in anhydrous methanol (Sigma–Aldrich, St. Louis, MO) according to the IUPAC (1987) standard method as described by Ruiz et al. (2008). Fatty acids quantification was performed using external standards with the aid of a calibration curve. Concentration range analysed (mg/100 g), calibration coefficients, linear regression coefficient, limit of detection (LOD) and limit of quantification (LOQ) calculated for each fatty acid methyl ester are shown in Table 4.

Myristic acid methyl ester, pentadecanoic acid methyl ester, palmitic acid methyl ester, palmitoleic acid methyl ester, heptadecanoic acid methyl ester, stearic acid methyl ester, oleic acid methyl ester, linoleic acid methyl ester, arachidic acid methyl ester, *cis*-11-eicosenoic acid methyl ester, linoleic acid methyl ester, heneicosenoic acid methyl ester, *cis*-11,14-eicosadienoic acid methyl ester, behenic acid methyl ester, lignoceric acid methyl ester with purity >98% GC and were purchased from Sigma–Aldrich (St Louis, MO). A DANI Master gas chromatograph (Dani Instrument SPA, Cologno Monzese, Milan, Italy) equipped with a PTV (programmed temperatures vaporiser), a flame ionisation detector (FID), and a SP2380 capillary column (Supelco, Bellefonte,

Table 3
Composition (coffee *Arabica* %, w/w) as indicated on the label of the analysed commercially available mixtures (C). Laboratory prepared mixtures (L) are also reported and their composition, as coffee *Arabica* percentage (w/w) given. Identification codes used to differentiate samples having different composition are given.

	Code name	Coffee *Arabica* (%, w/w)
Commercially available mixtures		
1	C1	100
2	C2	85
3	C3	80
4	C4	80
5	C5	80
6	C6	80
7	C7	70
8	C8	70
9	C9	70
10	C10	60
11	C11	50
12	C12	40
13	C13	30
Laboratory prepared mixtures		
1	L1	87
2	L2	73
3	L3	65
4	L4	50
5	L5	30
6	L6	20

Table 4
Concentration range analyzed (mg/100 g), calibration curves equations, linear regression coefficient, limit of detection (LOD) and limit of quantification (LOQ) are given.

Fatty acid	Concentration range analyzed (mg/100 g)	calibration curve equation	r^2	LOD (mg/100 g)	LOQ (mg/100 g)
14:0	2–10	$y = 0.0044 - 2.1556$	0.99	0.019	0.059
15:0	0.5–3	$y = 0.0028x - 2.3667$	0.99	0.010	0.032
16:0	1500–2000	$y = 0.0062x + 1.9944$	0.99	0.030	0.092
16:1	0.5–3	$y = 0.0017x - 0.1889$	0.99	0.008	0.024
17:0	2–10	$y = 0.0019x + 0.4278$	0.99	0.009	0.027
18:0	250–500	$y = 0.0039x + 0.4944$	0.99	0.019	0.057
18:1*n*–9c	300–600	$y = 0.0057x - 1.3778$	0.99	0.025	0.075
18:2*n*–6c	1500–3000	$y = 0.0019x + 0.4944$	0.99	0.009	0.028
20:0	50–150	$y = 0.0025x - 0.3167$	0.99	0.011	0.034
20:1	5–20	$y = 0.0024x - 1.0333$	0.99	0.011	0.035
18:3*n*–3	30–150	$y = 0.0023x - 0.5889$	0.99	0.010	0.032
21:0	0.5–3	$y = 0.0027x - 1.0667$	0.99	0.010	0.031
20:2	0.5–5	$y = 0.0027x - 0.0833$	0.99	0.010	0.030
22:0	5–20	$y = 0.0042x + 0.7222$	0.99	0.021	0.063
24:0	0.5–5	$y = 0.0038x - 1.2222$	0.99	0.015	0.045

PA), with dimensions of 50 m × 0.25 mm. I.D.; 0.25 μm film thickness, were used. Helium was used as carrier gas at a flow rate of 20 cm/s. The oven temperature program was as follows: 80 °C for 5 min, 5 °C/min to 165 °C for 5 min; and then 3 °C/min to 230 °C for 1 min. The split ratio was 1/60, and the FID temperature was set at 260 °C. The identifications of the peaks were made using an external analytical standard 37 Component FAME Mix (Supelco) by comparing the retention times with those of the pure standard components. Calibration of the fatty acids was carried out using the reference milk fat CRM (IRMM, Geel, Belgium). Fatty acids were determined and calculated as weight percentage (mg/100 g of fatty acids) as suggested by Molkentin (2009).

2.4. Statistical analysis

All determinations and experiments were performed in triplicate and the results are the average values of three determinations. Obtained data were analysed statistically with one-way analysis of variance (ANOVA) using the software XLSTAT 2006 (Addinsoft, Paris, France). The significance level (p) chosen was 0.05. In addition ANOVA statistical analysis was implemented with the Tukey *post hoc* test. Data were submitted to principal component analysis (PCA), which transforms the original set of variables into a smaller set of linear combinations and each discriminating factor is associated to a source (Motelay-Massei et al., 2007). PCA, which allowed the visualisation of sample trends (Martin et al., 2001), was followed by multiple linear regression (MLR) to quantify the content of *Arabica* in coffee blends according to the contribution of content of *Arabica* to each factor discriminated by principal component analysis (Clarysse et al., 2009; Pradelles et al., 2008).

3. Results and discussion

Fig. 1 shows fatty acids gas chromatograms of *Arabica* and Robusta coffees. Table 5 reports the fatty acid composition of the samples of roasted *Arabica* (A1, A2, A3, A4, A5, and A6) and *Robusta* (R1, R2, R3, R4, and R5). It can be observed that in both cultivars the following palmitic (C16:0), stearic (18:0), oleic (18:1n9c), linoleic (18:2n6c), arachidic (20:0) and linolenic (18:3n3c) acids are present at greater than 0.8%. The less represented fatty acids are: myristic (14:0), pentadecanoic (15:0), palmitoleic (16:1), heptadecanoic (17:0), eicosenoic (20:1), heneicosenoic (21:0), eicosadienoic (20:2), behenic (22:0), and lignoceric acids. The

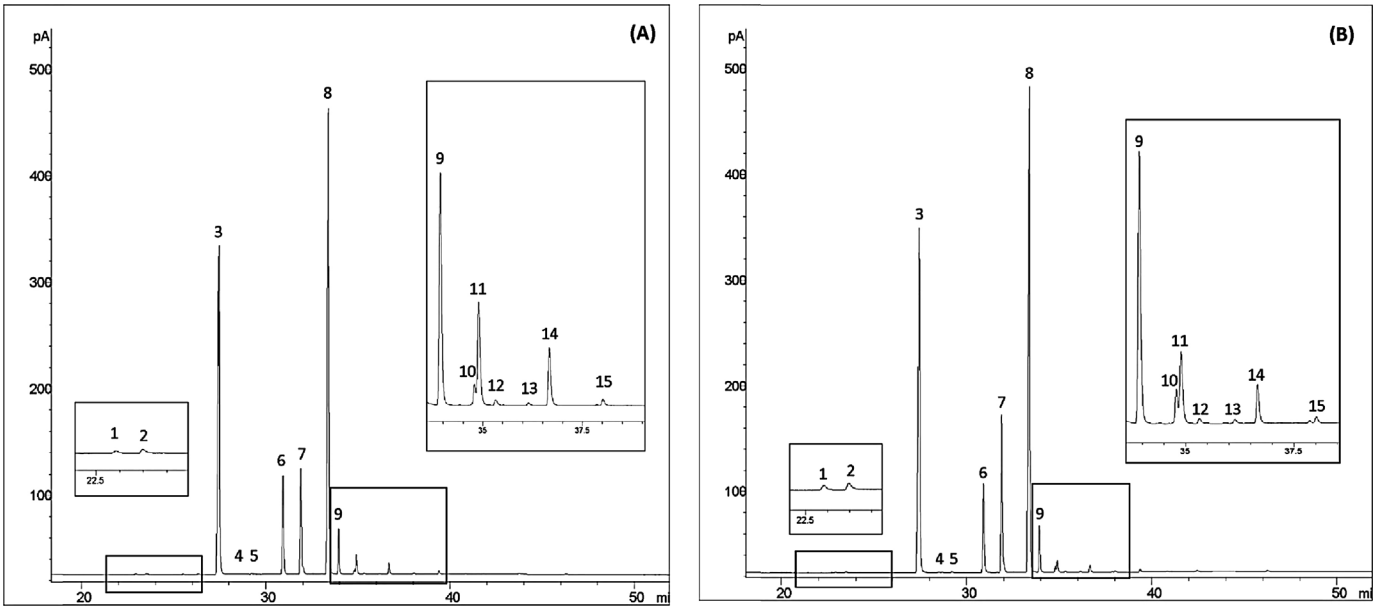

Fig. 1. Gas chromatograms of fatty acids in *arabica* (a) and *robusta* (b) varieties. 1, 14:0; 2, 15:0; 3, 16:0; 4, 16:1; 5, 17:0; 6, 18:0; 7, 18:1n9c; 8, 18:2n6c; 9, 20:0; 10, 20:1; 11, 18:3n3; 12, 21:0; 13, 20:2; 14, 22:0; 15, 24:0.

Table 5
Fatty acids (FA) composition of analysed roasted coffee of varieties *Arabica* (A) and *Canephora* (R).

FA (mg/100 g)	A1	A2	A3	A4	A5	A6
14:0	$65.4^{b,c} \pm 3.60$	$61.3^{c} \pm 0.69$	$77.9^{a,b,c} \pm 0.08$	$72.4^{a,b,c} \pm 3.55$	$65.8^{b,c} \pm 4.30$	$65.9^{b,c} \pm 4.03$
15:0	$29.6^{b} \pm 2.55$	$26.1^{b} \pm 2.84$	$31.6^{b} \pm 3.51$	$21.6^{b} \pm 2.62$	$80.2^{a} \pm 2.18$	$95.3^{b} \pm 3.54$
16:0	$34{,}930^{a} \pm 63.60$	$35{,}910^{a} \pm 37.2$	$36{,}933^{a} \pm 24.2$	$37{,}718^{a} \pm 42.5$	$36{,}641^{a} \pm 66.4$	$36{,}152^{a} \pm 88.0$
16:1	$26.9^{b,c} \pm 0.68$	$26.8^{b,c} \pm 0.69$	$30.7^{b} \pm 3.48$	$25.4^{b,c} \pm 1.38$	$12.7^{d} \pm 1.38$	$24.9^{b,c} \pm 2.45$
17:0	$114^{a,b} \pm 1.44$	$115^{a,b} \pm 2.93$	$115^{a,b} \pm 1.59$	$105^{b} \pm 2.64$	$115^{a,b} \pm 1.69$	$123^{a} \pm 1.04$
18:0	$5831^{d} \pm 37.5$	$6603^{a} \pm 49.9$	$7115^{a} \pm 17.4$	$6490^{b,c} \pm 20.2$	$6451^{b,c} \pm 67.1$	$6321^{c,d} \pm 49.3$
18:1*n*–9c	$7475^{e} \pm 24.8$	$7323^{e} \pm 8.19$	$7912^{e} \pm 8.84$	$7900^{e} \pm 10.7$	$7894^{e} \pm 38.0$	$7812^{e} \pm 7.78$
18:2*n*–6c	$45{,}840^{a} \pm 21.8$	$43{,}580^{b} \pm 53.9$	$41{,}389^{c,d} \pm 16.7$	$43{,}240^{b,c} \pm 43.3$	$44{,}318^{a,b} \pm 13.7$	$44{,}703^{a,b} \pm 32.7$
20:0	$2202^{b} \pm 4.79$	$2653^{a} \pm 14.5$	$2694^{a} \pm 16.1$	$1434^{d} \pm 8.30$	$1597^{c,d} \pm 12.9$	$1715^{c,d} \pm 10.4$
20:1	$311^{a} \pm 1.42$	$318^{b} \pm 11.5$	$277^{c,d} \pm 10.1$	$192^{b,c} \pm 2.76$	$202^{a,b} \pm 0.66$	$215^{a,b} \pm 3.34$
18:3*n*–3	$1463^{a} \pm 3.09$	$1301^{b} \pm 1.60$	$1306^{b} \pm 3.99$	$1440^{a} \pm 3.33$	$1423^{a} \pm 7.60$	$1417^{a} \pm 2.20$
21:0	$54.7^{a} \pm 3.49$	$69.4^{a} \pm 2.46$	$76.3^{a} \pm 2.16$	$68.4^{a} \pm 4.07$	$35.5^{a} \pm 2.56$	$42.9^{a} \pm 3.06$
20:2	$46.7^{a} \pm 1.34$	$41.9^{a,b,c} \pm 1.67$	$35.7^{a,b,c,d,e} \pm 1.36$	$30.1^{c,d,e} \pm 1.32$	$26.8^{e} \pm 0.66$	$28.7^{d,e} \pm 2.00$
22:0	$437^{b} \pm 3.20$	$602^{a} \pm 5.75$	$657^{a} \pm 6.72$	$248^{d,e} \pm 4.03$	$248^{d,e} \pm 5.50$	$272^{d,e} \pm 2.70$
24:0	$76.5^{c,d} \pm 1.36$	$254^{a,b} \pm 1.78$	$268^{a} \pm 1.28$	$88.5^{c,d} \pm 1.68$	$50.8^{d} \pm 0.74$	$70.7^{d} \pm 1.79$
ΣSFA	$43{,}711^{c} \pm 143$	$46{,}293^{b,c} \pm 160$	$47{,}936^{a,b,c} \pm 117$	$46{,}224^{a,b,c} \pm 149$	$45{,}204^{a,b,c} \pm 145$	$44{,}752^{b,c} \pm 200$
ΣMUFA	$7813^{e} \pm 24.0$	$7668^{e} \pm 69.6$	$8219^{e} \pm 19.6$	$8117^{e} \pm 6.62$	$8108^{e} \pm 37.3$	$8052^{e} \pm 70.7$
ΣPUFA	$47{,}304^{a} \pm 210$	$44{,}880^{c,d} \pm 153$	$42{,}695^{a,b} \pm 193$	$44{,}680^{b,c} \pm 146$	$45{,}741^{a,b} \pm 121$	$46{,}120^{c,d} \pm 133$
∑SFA/∑UFA	$0.79^{c} \pm 0.79$	$0.88^{a,b,c} \pm 0.02$	$0.94^{b,c} \pm 0.04$	$0.88^{a,b,c} \pm 0.02$	$0.84^{b,c} \pm 0.02$	$0.83^{b,c} \pm 0.01$
ΣMUFA/ΣSFA	$0.18^{b} \pm 0.01$	$0.17^{b} \pm 0.01$	$0.17^{b} \pm 0.01$	$0.18^{b} \pm 0.01$	$0.18^{b} \pm 0.01$	$0.18^{b} \pm 0.01$
18:0/18:1*n*–9c	$0.78^{b} \pm 0.01$	$0.90^{a} \pm 0.02$	$0.95^{a} \pm 0.01$	$0.82^{b} \pm 0.01$	$0.82^{b} \pm 0.01$	$0.82^{b} \pm 0.01$

FA (mg/100 g)	R1	R2	R3	R4	R5
14:0	$81.5^{a,b,c} \pm 4.56$	$92.3^{a} \pm 1.77$	$87.9^{a,b} \pm 3.81$	$87.6^{a} \pm 4.73$	$93.6^{a,b} \pm 2.10$
15:0	$29.9^{b} \pm 0.35$	$28.9^{b} \pm 0.11$	$30.0^{b} \pm 2.74$	$22.6^{b} \pm 3.54$	$20.1^{b} \pm 0.01$
16:0	$36{,}404^{a} \pm 186$	$36{,}047^{a} \pm 143$	$34{,}279^{a} \pm 171$	$34{,}912^{a} \pm 143$	$35{,}973^{a} \pm 171$
16:1	$23.9^{b,c} \pm 2.35$	$19.9^{c,d} \pm 0.60$	$19.5^{c,d} \pm 2.70$	$24.4^{a} \pm 4.14$	$45.5^{b,c} \pm 3.42$
17:0	$77.6^{c} \pm 0.19$	$77.8^{c} \pm 3.23$	$77.6^{c} \pm 0.96$	$76.1^{c} \pm 1.47$	$82.3^{c} \pm 1.43$
18:0	$6690^{a,b,c} \pm 12.4$	$6783^{a,b,c} \pm 27.5$	$6943^{a,b} \pm 24.3$	$6742^{a,b,c} \pm 24.26$	$6575^{a,b,c} \pm 6.76$
18:1*n*–9c	$11{,}939^{c} \pm 0.01$	$13{,}015^{a} \pm 0.39$	$12{,}305^{b,c} \pm 0.12$	$12{,}773^{a,b} \pm 0.20$	$10{,}959^{d} \pm 0.09$
18:2*n*–6c	$38{,}465^{e} \pm 245$	$38{,}405^{a} \pm 274$	$40{,}119^{b,c} \pm 161$	$40{,}605^{a,b} \pm 147$	$41{,}335^{d} \pm 153$
20:0	$2649^{c} \pm 161$	$2173^{a} \pm 98.0$	$2610^{b,c} \pm 95.0$	$1756^{a,b} \pm 46.4$	$1836^{d} \pm 50.9$
20:1	$400^{e} \pm 13.4$	$436^{e} \pm 10.4$	$435^{d,e} \pm 18.8$	$299^{d} \pm 9.44$	$262^{c,d} \pm 7.55$
18:3*n*–3	$672^{d,e} \pm 8.77$	$661^{e} \pm 9.70$	$724^{c,d,e} \pm 26.1$	$745^{c,d} \pm 18.5$	$789^{e} \pm 7.27$
21:0	$62.5^{a} \pm 6.90$	$51.5^{a} \pm 6.03$	$66.9^{a} \pm 0.20$	$45.8^{a} \pm 3.05$	$52.2^{a} \pm 5.33$
20:2	$40.9^{a,b,c,d} \pm 0.01$	$39.3^{a,b,c,d,e} \pm 0.01$	$43.3^{a,b} \pm 0.00$	$33.4^{b,c,d,e} \pm 0.01$	$40.5^{a,b,c,d} \pm 0.02$
22:0	$408^{b} \pm 36.2$	$305^{c,d} \pm 4.26$	$390^{b,c} \pm 8.70$	$198^{c} \pm 6.85$	$207^{c} \pm 8.33$
24:0	$273^{a} \pm 12.2$	$219^{b} \pm 2.82$	$259^{a,b} \pm 5.88$	$108^{c} \pm 4.44$	$70.0^{c,d} \pm 4.83$
ΣSFA	$46{,}646^{a,b} \pm 104$	$45{,}750^{b,c} \pm 150$	$44{,}713^{b,c} \pm 95.5$	$43{,}926^{b,c} \pm 70.3$	$44{,}885^{b,c} \pm 64.2$
ΣMUFA	$12{,}363^{c} 123$	$13{,}471^{a} \pm 119$	$12{,}759^{a,b} \pm 114$	$13{,}097^{d} \pm 134$	$11{,}267^{a,b} \pm 100$
ΣPUFA	$39{,}137^{e} \pm 148$	$39{,}066^{e} \pm 186$	$40{,}842^{d,e} \pm 102$	$41{,}350^{d} \pm 177$	$42{,}124^{d} \pm 127$
∑SFA/∑UFA	$0.91^{a,b} \pm 0.01$	$0.87^{a,b} \pm 0.03$	$0.83^{a,b,c} \pm 0.05$	$0.81^{b,c} \pm 0.02$	$0.84^{c} \pm 0.02$
ΣMUFA/ΣSFA	$0.27^{a} \pm 0.01$	$0.29^{a} \pm 0.01$	$0.29^{a} \pm 0.02$	$0.30^{a} \pm 0.02$	$0.25^{a} \pm 0.01$
18:0/18:1*n*–9c	$0.55^{c,d} \pm 0.01$	$0.52^{d} \pm 0.01$	$0.56^{c,d} \pm 0.00$	$0.53^{d} \pm 0.01$	$0.60^{c} \pm 0.01$

[a–e] Different letters indicate statistically significant differences between different varieties for $p \leq 0.05$.

summation of the saturated fatty acids (SFA) including the polyunsaturated fatty acids (PUFA) constitutes about 80.0% of the total fatty acid amount.

Palmitic acid (C16:0) was the most prevalent among the SFA, with an average percentage in the two cultivars *Arabica* and *Canephora* of 36.18% and 36.08%, respectively. Oleic acid (*cis*18:1*n*–9), the most abundant MUFA was present in *Robusta* and *Arabica* at percentages of 12.09% and 7.71%, respectively. The average concentration of linoleic acid (*cis*18:2*n*–6) in *Arabica* was 43.60% and in *Robusta* was 39.26%. α-Linolenic acid (*cis*18:3*n*–3) was observed in a greater amount in the *Arabica* (average value 1.37%) than *Robusta* (average value of 0.69%). These observations agree with other reported data (Martin et al., 2001). The application of ANOVA to the individual fatty acids in the two cultivars showed significant differences ($p < 0.05$) for 17:0, *cis*18:1*n*–9 and *cis*18:3*n*–3.

Table 6 reports the fatty acids content in the analysed mixtures used to build the calibration curves. The SFA concentration and the ratio between saturated fatty acids and unsaturated fatty acids (∑SFA/∑UFA) do not indicate any significant difference in the analysed mixtures. On the other hand, the summation of monounsaturated fatty acids (ΣMUFA), the summation of polyunsaturated fatty acids (ΣPUFA), the *cis*18:3*n*–3 concentration and the ratio between 18:0/*cis*18:1*n*–9 and ΣMUFA/ΣSFA, showed statistically significant differences in *Arabica*/*Robusta* mixtures. Fig. 2 shows a PCA scores plot. The first component explains 97.56% of the total variation in the data and is associated with the ∑MUFA, *cis*18:3*n*–3 and ∑MUFA/∑SFA and 18:0/*cis*18:1*n*–9 ratios. Samples of *Arabica* were positioned to the right of the plot, and *Robusta* to the left. Mixtures with known composition of *Arabica* and *Robusta*, positioned themselves in an orderly manner between the 100% *Robusta* and 100% *Arabica* samples.

Fig. 3 reports the variation of ΣMUFA, the *cis*18:3*n*–3 content, the 18:0/*cis*18:1*n*–9 and ∑MUFA/∑SFA ratios, with respect to the *Arabica* coffee percentage present in the mixtures. It can be observed that there is always a linear correlation: the amount of *cis*18:3*n*–3 and the18:0/*cis*18:1*n*–9 ratio increases with the increase of the *Arabica* coffee percentage, while ΣMUFA and the ratio ∑MUFA/∑SFA decrease with increasing *Arabica* percentage.

Data were also analysed using multiple linear regression (Fig. 4), and a linear dependence between the markers discriminated by PCA and the content of *Arabica* was observed. The linear regression coefficient was 0.98.

Table 6
Fatty acids (FA) composition of the roasted coffee mixtures with a known coffee *Arabica* composition.

FA (mg/100 g)	MR	M1	M2	M3	M4	M5
14:0	$103^{a} \pm 4.99$	$91.7^{a,b} \pm 3.53$	$88.4^{b} \pm 3.61$	$80.6^{b,c} \pm 8.66$	$82.6^{b,c} \pm 4.56$	$84.3^{b,c} \pm 4.27$
15:0	$22.8^{a} \pm 1.26$	$21.7^{a} \pm 2.11$	$18.3^{a} \pm 2.85$	$11.0^{a} \pm 0'.72$	$12.0^{a} \pm 2.94$	$22.8^{a} \pm 0.70$
16:0	$35{,}403^{a} \pm 184$	$34{,}707^{a} \pm 152$	$35{,}269^{a} \pm 158$	$36{,}117^{a} \pm 160$	$35{,}738^{a} \pm 210$	$35{,}430^{a} \pm 156$
16:1	$20.5^{a} \pm 1.37$	$19.5^{a} \pm 1.39$	$26.9^{a} \pm 1.37$	$22.5^{a} \pm 2.78$	$23.9^{a} \pm 0.10$	$27.8^{a} \pm 0.68$
17:0	$83.60^{f} \pm 0.75$	$82.09^{f} \pm 0.69$	$87.57^{e,f} \pm 0.77$	$93.28^{d,e,f} \pm 0.14$	$96.96^{c,d,e} \pm 1.13$	$96.47^{c,d,e} \pm 1.91$
18:0	$6764^{a} \pm 19.0$	$6815^{a} \pm 72.6$	$6780^{a} \pm 93.4$	$6389^{c} \pm 54.7$	$6446^{b,c} \pm 48.2$	$6648^{a,b} \pm 74.1$
18:1*n*–9c	$12{,}045^{a} \pm 0.48$	$11{,}567^{a} \pm 14.8$	$10{,}968^{b} \pm 99.8$	$9933^{c} \pm 12.8$	$9589^{c,d} \pm 8.44$	$9402^{d} \pm 36.8$
18:2*n*–6c	$40{,}458^{f} \pm 61.0$	$41{,}577^{e} \pm 96.5$	$41{,}581^{e} \pm 99.5$	$42{,}847^{d} \pm 40.4$	$43{,}498^{b,c,d} \pm 39.9$	$42{,}993^{c,d} \pm 64.6$
20:0	$2041^{a,b} \pm 12.3$	$2072^{a,b} \pm 8.08$	$2107^{a,b} \pm 14.8$	$1658^{c} \pm 21.9$	$1667^{c} \pm 60.1$	$1944^{b} \pm 36.6$
20:1	$347^{a} \pm 1.24$	$337^{a} \pm 13.9$	$321^{a} \pm 24.1$	$240^{c,d} \pm 24.2$	$241^{b,c} \pm 29.5$	$268^{b,c} \pm 6.68$
18:3*n*–3	$737^{a} \pm 3.93$	$829^{b} \pm 2.07$	$900^{c} \pm 11.9$	$1037^{d} \pm 19.2$	$1117^{e} \pm 0.48$	$1144^{f} \pm 9.33$
21:0	$50.7^{a} \pm 2.07$	$44.8^{a} \pm 3.63$	$46.5^{a} \pm 9.08$	$41.8^{a,b} \pm 2.05$	$29.1^{b,c} \pm 6.05$	$44.8^{a} \pm 1.41$
20:2	$39.9^{a} \pm 1.31$	$35.5^{a} \pm 1.37$	$40.5^{a} \pm 1.30$	$38.1^{a} \pm 3.31$	$34.8^{a} \pm 0.02$	$34.9^{a} \pm 5.47$
22:0	$254^{e,f} \pm 1.45$	$262^{d,c} \pm 17.6$	$296^{c,d} \pm 29.35$	$225^{e,f} \pm 28.8$	$216^{e,f} \pm 14.3$	$320^{b,a} \pm 14.5$
24:0	$131^{a} \pm 5.97$	$119^{a} \pm 6.76$	$122^{a} \pm 13.3$	$58.1^{c,d} \pm 10.3$	$72.3^{b} \pm 11.0$	$96.9^{a,b} \pm 11.9$
ΣSFA	$44{,}831^{a} \pm 156$	$44{,}193^{a} \pm 371$	$44{,}796^{a} \pm 126$	$44{,}665^{a} \pm 389$	$44{,}348^{a} \pm 197$	$44{,}664^{a} \pm 244$
ΣMUFA	$12{,}413^{f} \pm 48.3$	$11{,}923^{f} \pm 27.4$	$11{,}316^{e,f} \pm 36.4$	$10{,}196^{e,f} \pm 55.1$	$9854^{c,d,e} \pm 15.21$	$9698^{d,e,f} \pm 42.3$
ΣPUFA	$41{,}194^{a} \pm 166$	$42{,}407^{a} \pm 95.5$	$42{,}480^{a,b} \pm 114$	$43{,}884^{a,b} \pm 143$	$44{,}615^{a,b,c} \pm 33.9$	$44{,}137^{a,b,c} \pm 75.9$
∑SFA/∑UFA	$0.84^{a} \pm 0.05$	$0.81^{a} \pm 0.01$	$0.83^{a} \pm 0.04$	$0.83^{a} \pm 0.01$	$0.81^{a} \pm 0.01$	$0.83^{a} \pm 0.01$
ΣMUFA/ΣSFA	$0.28^{a} \pm 0.01$	$0.27^{a,b} \pm 0.03$	$0.25^{a,b,c} \pm 0.01$	$0.23^{a,b,c} \pm 0.01$	$0.22^{b,c,d} \pm 0.00$	$0.22^{b,c,d} \pm 0.01$
18:0/18:1*n*–9c	$0.56^{h} \pm 0.01$	$0.59^{g,h} \pm 0.01$	$0.62^{f,g} \pm 0.02$	$0.64^{e,f,g} \pm 0.03$	$0.67^{d,e,f} \pm 0.00$	$0.69^{d,e} \pm 0.01$

FA (mg/100 g)	M6	M7	M8	M9	MA
14:0	$81.6^{b,c} \pm 3.47$	$70.4^{c} \pm 4.56$	$67.4^{c} \pm 4.27$	$69.4^{c} \pm 4.15$	$70.9^{c} \pm 7.74$
15:0	$18.0^{a} \pm 2.45$	$20.3^{a} \pm 294$	$15.5^{a} \pm 0.70$	$18.6^{a} \pm 2.07$	$20.1^{a} \pm 3.10$
16:0	$35{,}844^{a} \pm 106$	$35{,}144^{a} \pm 138$	$36{,}231^{a} \pm 191$	$35{,}210^{a} \pm 0131$	$35{,}181^{a} \pm 106$
16:1	$31.7^{a} \pm 0.10$	$26.1^{a} \pm 1.24$	$30.3^{a} \pm 0.68$	$26.6^{a} \pm 0.24$	$25.9^{a} \pm 0.33$
17:0	$99.6^{b,c,d} \pm 1.13$	$107^{a,d,c} \pm 1.24$	$109.5^{a,b} \pm 1.41$	$111^{a,b} \pm 2.45$	$114^{a} \pm 1.64$
18:0	$6376^{c} \pm 47.2$	$6796^{a} \pm 53.6$	$6338^{c} \pm 74.0$	$6761^{a} \pm 74.4$	$6786^{a} \pm 96.3$
18:1*n*–9c	$9176^{d,e} \pm 8.44$	$8796^{e} \pm 3.58$	$8208^{f} \pm 10.2$	$8112^{f} \pm 56.3$	$7978^{f} \pm 51.2$
18:2*n*–6c	$43{,}830^{a,b,c} \pm 39.9$	$43{,}616^{a,b,c,d} \pm 24.5$	$44{,}474^{a} \pm 34.7$	$44{,}316^{a,b} \pm 17.8$	$44{,}395^{a,b} \pm 88.2$
20:0	$1625^{c} \pm 30.1$	$2124^{a} \pm 23.7$	$1585^{c} \pm 36.6$	$2142^{a} \pm 22.9$	$2127^{a} \pm 27.5$
20:1	$242^{b,c} \pm 6.66$	$274^{b} \pm 6.17$	$208^{d} \pm 7.09$	$256^{b,c} \pm 0.69$	$251^{b,c} \pm 0.44$
18:3*n*–3	$1179^{g} \pm 0.48$	$1241^{h} \pm 0.97$	$1326^{i} \pm 0.78$	$1351^{j} \pm 0.69$	$1389^{k} \pm 0.58$
21:0	$24.1^{a} \pm 6.50$	$42.6^{a,b} \pm 5.42$	$28.6^{b,c} \pm 1.41$	$44.8^{a} \pm 5.03$	$51.5^{a} \pm 4.06$
20:2	$32.9^{a} \pm 5.47$	$34.3^{a} \pm 2.00$	$32.5^{a} \pm 2.58$	$31.8^{a} \pm 3.06$	$36.9^{a} \pm 2.09$
22:0	$209.1^{f} \pm 14.2$	$359^{a,b} \pm 14.6$	$234^{e,f} \pm 15.5$	$395.6^{a} \pm 11.0$	$398^{a} \pm 9.07$
24:0	$50.8^{c,d} \pm 8.78$	$103^{a,b} \pm 11.0$	$106^{d} \pm 11.9$	$103^{a,b} \pm 15.9$	$103^{a,b} \pm 17.3$
ΣSFA	$44{,}310^{a} \pm 155$	$44{,}747^{a} \pm 138$	$44{,}629^{a} \pm 127$	$44{,}837^{a} \pm 129$	$44{,}832^{a} \pm 117$
ΣMUFA	$9450^{b,c,d} \pm 15.2$	$9095^{b,c} \pm 17.3$	$8446^{a,b} \pm 14.5$	$8395^{a} \pm 16.7$	$8255^{a} \pm 5.24$
ΣPUFA	$45{,}010^{b,c,d} \pm 33.9$	$44{,}858^{c,d,e} \pm 75.9$	$45{,}800^{e,f} \pm 35.7$	$45{,}667^{d,e} \pm 30.32$	$45{,}783^{f} \pm 28.7$
∑SFA/∑UFA	$0.81^{a} \pm 0.01$	$0.83^{a} \pm 0.01$	$0.82^{a} \pm 0.01$	$0.83^{a} \pm 0.01$	$0.83^{a} \pm 0.03$
ΣMUFA/ΣSFA	$0.21^{c,d} \pm 0.02$	$0.20^{a,b,c} \pm 0.03$	$0.19^{d} \pm 0.02$	$0.19^{d} \pm 0.01$	$0.18^{d} \pm 0.01$
18:0/18:1*n*–9c	$0.71^{c,d} \pm 0.01$	$0.77^{b,c} \pm 0.02$	$0.77^{b} \pm 0.01$	$0.83^{a} \pm 0.02$	$0.85^{a} \pm 0.04$

[a–k] Different letters indicate statistically significant differences between different content of coffee *Arabica* for $p \leq 0.05$.

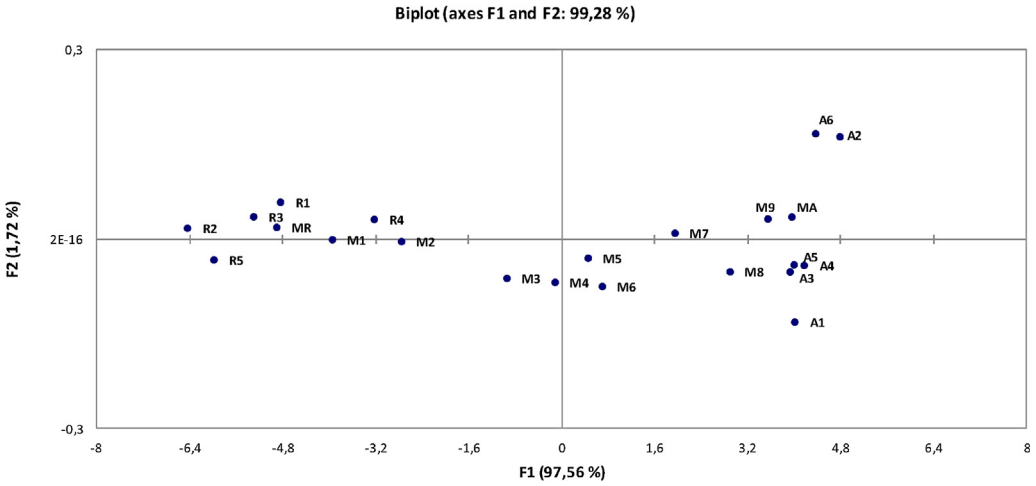

Fig. 2. Scores plot for the first principal components.

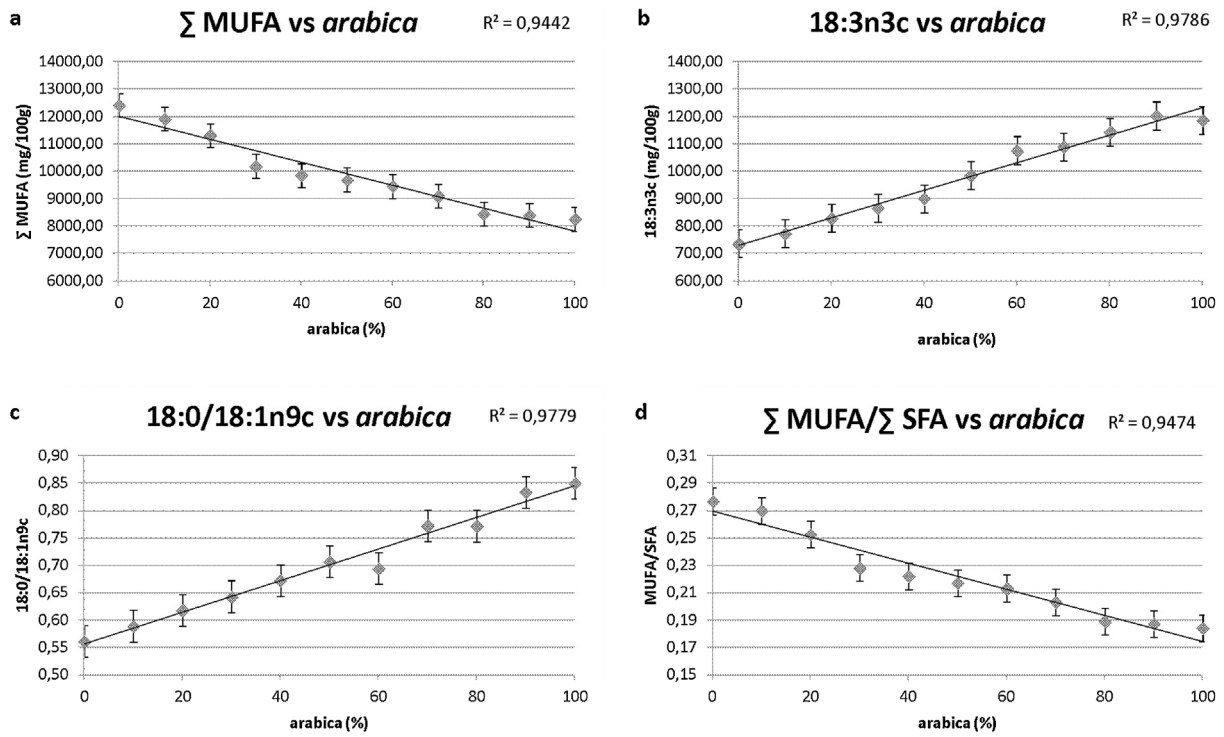

Fig. 3. Linear regression curves between the main components selected by PCA [∑MUFA (a), *cis*18:3*n*–3 (b), C18:0/*cis*18:1*n*–9 (c), ∑MUFA/∑SFA (d)], and the content of *Arabica* in coffee mixtures.

Table 7 shows the fatty acids composition of the commercial coffee samples analysed, labelled from C1 to C13. The information regarding the composition was reported on the label by the producer in term of percentage of coffee *Arabica*. Table 7 reports also the fatty acids compositions of the laboratory prepared mixtures, labelled from L1 to L6. In all the analysed samples, the fatty acids fractions 16:0, 18:0, *cis*18:1*n*–9, *cis*18:2*n*–6, 20:0 and *cis*18:3*n*–3, represented about 95% of the identified fatty acids. In order to estimate the content of coffee *Arabica* in the laboratory coffee blends and in the coffee mixture purchased from the local market, two quantitative methods were proposed. Using the average model (AM), the ∑MUFA, *cis*18:3*n*–3, ∑MUFA/∑SFA and 18:0/*cis*18:1*n*–9 values calculated for each sample were interpolated to the respective calibration curves and the percentage of *Arabica* was determined as the average of four determinations. Using the multiple linear regression model (MLRM), the percentage of *Arabica* was directly obtained by inserting the value of the four markers estimated for each samples in the equation of the curve showed in Fig. 4.

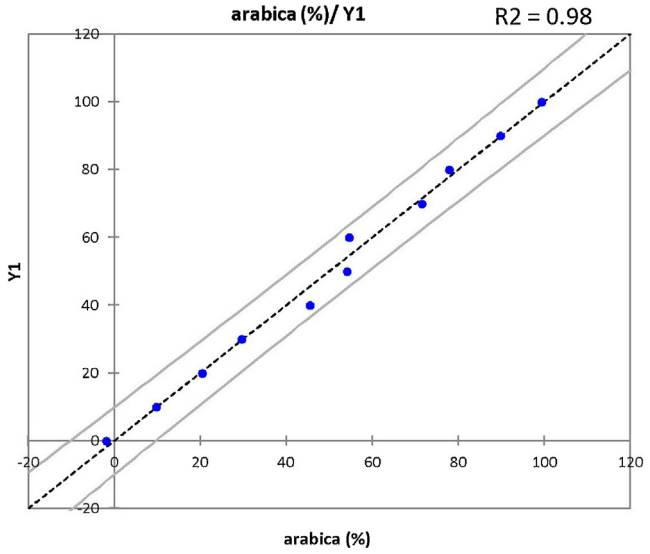

Fig. 4. Multiple linear regression curve between the main components selected by PCA (∑MUFA, *cis*18:3*n*–3, ∑MUFA/∑SFA and 18:0/*cis*18:1*n*–9 values) and the content of *Arabica* in coffee mixtures.

Table 8 shows the percentages of *Arabica* quantified in coffee samples prepared in the laboratory (L1–L6) and in those obtained from the local market (C1–C13) by applying the proposed models. By applying the AM model for mixtures L1, L2, L3, L4, L5 and L6 a percentage of *Arabica* was estimated of 87.47%, 70.71%, 67.49%, 55.29%, 34.17% and 22.43%, respectively. A deviation with respect to the real value of 0.54%, −3.13%, 3.84%, 10.59%, 13.89% and 12.15%, respectively was observed. By applying MLRM model for mixtures L1, L2, L3, L4, L5 and L6 a percentage of *Arabica* of 81.85%, 74.14%, 100.30%, 40.89%, 28.03% and 13.16%, respectively was estimated, with deviations from the real value of 5.92%, −1.56%, −54.30%, 18.22%, 6.58% and 34.18%, respectively.

It was also observed that in coffee samples that contained percentage of *Arabica* up to 60% (L1, L2 and L3) the AM model gave results closer to the real composition when compared to the results obtained from MRLM model. In both mathematical models the error increase was directly proportional to the amount of *Robusta* in the mixture but again, in this case, the AM model was more predictive and accurate than the MRLM model. The greater variability of the fatty acid composition in the *Robusta* variety could explain the reduced applicability of the MRLM model for coffee blends that contained more than 50% (w/w) of *Robusta* (L4, L5 and L6).

By applying the AM model to the commercial coffee blends, it was observed that there was a maximum deviation with respect to the percentage of *Arabica* declared by the producer of 11% for

Table 7
Fatty acids (FA) composition of the analysed coffee mixtures available from the local market.

FA (mg/100 g)	C1	C2	C3	C4	C5	C6	C7
14:0	62.9^{a} ± 1.25	66.9^{a} ± 1.44	76.3^{a} ± 7.84	82.1^{a} ± 1.47	73.3^{a} ± 2.74	88.7^{a} ± 1.43	76.5^{a} ± 0.71
15:0	25.1^{a,b,c,d} ± 1.34	27.1^{a,b} ± 1.68	30.1^{a,b} ± 1.24	31.1^{a,b} ± 1.42	27.5^{a,b} ± 2.08	31.1^{a,b} ± 1.41	26.6^{a,b,c} ± 0.70
16:0	34,534^{b} ± 78.5	34,188^{b} ± 144	36,632^{a,b} ± 188	35,703^{a,b} ± 122	36,487^{a,b} ± 201	35,346^{a,b} ± 250	33,630^{b} ± 108
16:1	25.9^{a} ± 0.76	25.9^{a} ± 3.46	26.3^{a} ± 2.75	27.4^{a} ± 1.38	24.4^{a} ± 1.34	22.9^{a} ± 2.07	24.9^{a} ± 0.69
17:0	106^{a,b} ± 1.05	103^{a,b} ± 2.19	102^{a,b} ± 2.23	105^{a,b} ± 2.19	103^{b} ± 1.64	96.9^{b} ± 1.49	105^{a,b} ± 5.90
18:0	7091^{a,b,c,d} ± 42.8	7191^{a,b,c} ± 45.4	7032^{a,b,c,d} ± 54.3	6921^{a,b,c,d} ± 45.1	7067^{a,b,c,d} ± 95.3	6837^{a,b,c,d,e} ± 5.37	7551^{a} ± 14.7
18:1*n*–9c	8382^{e,f,g} ± 97.9	9035^{c,d,e,f} ± 23.9	8704^{d,e,f,g} ± 40.1	8611^{d,e,f,g} ± 9.41	8591^{d,e,f,g} ± 2.72	9610^{b,c} ± 14.9	9210^{c,d} ± 1.35
18:2*n*–6c	44,037^{a,b,c} ± 186	42,922^{a,b,c,d,e} ± 197	40,919^{f} ± 177	43,668^{e,f} ± 166	41,279^{a,b,c,d} ± 125	43,317^{a,b,c,d} ± 192	42,734^{a,b,c,d,e,f} ± 6.66
20:0	2475^{a} ± 104	2626^{a} ± 4.54	2753^{a} ± 129	1809^{a} ± 13.1	2766^{a} ± 9.67	1842^{a} ± 9.61	2705^{a} ± 9.30
20:1	309^{a} ± 5.86	328^{a} ± 4.78	320^{a} ± 4.14	213^{b} ± 2.05	317^{a} ± 14.0	234^{b} ± 0.04	318^{a} ± 2.03
18:3*n*–3	1374^{a} ± 1.25	1319^{a,b}20.1	1270^{a,b,c} ± 17.5	1313^{a,b} ± 13.2	1271^{a,b,c} ± 4.24	1121^{d,e} ± 11.85	1339^{a,b} ± 2.75
21:0	57.8^{b,c} ± 3.10	69.9^{a,b} ± 1.40	76.3^{a} ± 3.28	43.3^{c,d} ± 1.39	75.3^{a,b} ± 0.57	39.9^{c,d,e} ± 0.70	71.5^{a,b} ± 2.09
20:2	37.3^{a,b} ± 0.55	40.5^{a,b} ± 1.34	34.3^{a} ± 2.00	25.9^{c,d} ± 1.99	37.6^{a} ± 1.05	31.1^{a,b,c} ± 2.66	41.0^{a,b} ± 0.67
22:0	493^{a,b}±6.40	529^{a,b} ± 1.47	569^{a} ± 10.3	282^{c} ± 4.15	579^{a} ± 1.63	247^{c,d,e} ± 0.72	515^{a,b} ± 0.00
24:0	181^{b} ± 5.07	225^{a,b} ± 7.37	257^{a} ± 2.29	91.1^{c,d} ± 1.47	257^{a} ± 2.53	97.4^{c} ± 2.90	221^{a,b} ± 2.96
ΣSFA	45,001^{b} ± 135	45,027^{b} ± 149	47,498^{a} ± 75.5	45,036^{b} ± 245	47,408^{a} ± 163	44,595^{b} ± 230	44,875^{b} ± 135
ΣMUFA	8718^{f,g,h} ± 92.8	9389^{c,d,e,f}±32.2	9050^{e,f,g,h} ± 41.5	8851^{e,f,g,h} ± 5.97	8933^{e,f,g,h} ± 2.84	9868^{b,c,d} ± 17.0	9552^{c,d,e} ± 0.02
ΣPUFA	45,411^{a,b} ± 128	44,241^{a,b,c,d,e} ± 119	42,189^{a,b,c,d,e} ± 196	44,981^{a,b,c,d} ± 177	42,549^{e,f} ± 127	44,438^{a,b,c,d,e} ± 207	44,074^{b,c,d,e,f} ± 3.24
∑SFA/∑UFA	0.83^{b,c} ± 0.01	0.84^{a,b,c} ± 0.01	0.93^{a} ± 0.02	0.84^{a,b,c} ± 0.01	0.92^{a,b} ± 0.05	0.82^{c} ± 0.01	0.84^{a,b,c} ± 0.01
ΣMUFA/ΣSFA	0.19^{f,g} ± 0.01	0.21^{c,d,e,f,g} ± 0.03	0.19^{f,g} ± 0.01	0.20^{d,e,f,g} ± 0.02	0.19^{f,g} ± 0.01	0.22^{a,b,c,d} ± 0.01	0.21^{b,c,d,e,f,g} ± 0.01
18:0/18:1*n*–9c	0.85^{a} ± 0.01	0.80^{b,c,d} ± 0.02	0.81^{b,c} ± 0.01	0.80^{b,c} ± 0.01	0.82^{a,b} ± 0.01	0.71^{h,i} ± 0.02	0.82^{a,b} ± 0.01

FA (mg/100 g)	C8	C9	C10	C11	C12	C13
14:0	70.9^{a} ± 2.84	64.1^{a} ± 1.83	100^{a} ± 1.29	83.1^{a} ± 1.22	84.7^{a} ± 0.71	91.2^{a} ± 1.68
15:0	29.1^{a,b} ± 2.82	35.0^{a} ± 0.54	21.0^{b,c,d,e} ± 1.56	15.1^{d,e} ± 0.76	28.1^{a,b} ± 0.02	22.6^{b,c,d,e} ± 0.72
16:0	35,378^{a,b} ± 147	33,587^{b} ± 143	35,097^{a,b} ± 185	34,818^{b} ± 199	33,658^{b} ± 131	35,181^{a,b} ± 124
16:1	27.4^{a} ± 6.91	27.8^{a} ± 8.24	25.9^{a} ± 3.14	25.4^{a} ± 2.24	25.9^{a} ± 2.07	27.8^{a} ± 4.82
17:0	105^{a,b} ± 0.70	99.2^{b} ± 1.77	102^{b} ± 3.04	102^{b} ± 1.08	98.6^{b} ± 6.63	97.5^{b} ± 1.53
18:0	6961^{a,b,c,d} ± 111	7345^{a,b} ± 104	6502^{c,d,e} ± 157	6813^{a,b,c,d,e} ± 137	7349^{a,b} ± 10.5	6610^{b,c,d,e} ± 132
18:1*n*–9c	8819^{d,e,f} ± 47.8	9614^{b,c} ± 42.1	8318^{f,g} ± 59.4	9165^{c,d} ± 70.2	9663^{b,c} ± 40.4	9639^{b,c} ± 76.6
18:2*n*–6c	43,977^{a,b,c,d} ± 132	42,291^{c,d,e,f} ± 177	44,334^{a,b} ± 121	43,978^{a,b,c,d} ± 133	42,662^{b,c,d,e,f} ± 109	43,627^{a,b,c,d} ± 136
20:0	1725^{a} ± 3.67	2794^{a} ± 2.05	1763^{a} ± 12.5	1877^{b} ± 15.6	2766^{a} ± 1.45	1857^{b} ± 153
20:1	232^{b} ± 8.18	331^{a} ± 6.24	209^{b} ± 18.5	238^{b} ± 18.4	332^{a} ± 4.73	236^{b} ± 14.9
18:3*n*–3	1325^{a,b} ± 22.4	1228^{b,c,d} ± 18.9	1131^{d,e} ± 10.3	1170^{c,d} ± 22.3	1236^{b,c,d} ± 15.77	1040^{e,f} ± 20.3
21:0	45.3^{c,d} ± 2.77	76.1^{a,b} ± 1.62	36.9^{d,e,f} ± 10.0	24.1^{e,f} ± 6.24	70.9^{a,b} ± 1.40	20.7^{f} ± 4.19
20:2	29.2^{c} ± 1.34	42.2^{a,b} ± 3.22	37.7^{a,b,c} ± 0.03	36.3^{c,d,e} ± 0.51	42.4^{a} ± 0.10	40.9^{a} ± 8.67
22:0	292^{c} ± 17.1	499^{a,b} ± 7.34	229^{c,d,e} ± 8.70	233^{c} ± 6.22	436^{b} ± 7.55	207^{c,d,e} ± 18.6
24:0	88.6^{c,d,e} ± 7.01	215^{a,b} ± 5.24	24.6^{f} ± 0.76	45.1^{d,e,f} ± 0.98	206^{b} ± 5.19	33.0^{f} ± 0.01
ΣSFA	44,665^{b} ± 113	45,120^{b} ± 0.06	43,854^{b} ± 85.4	43,996^{b} ± 186	44,670^{b} ± 199	44,099^{b} ± 150
ΣMUFA	9079^{d,e,f,g,h} ± 62.9	9972^{b,c} ± 0.04	8552^{g,h} ± 62.6	9428^{c,d,e,f} ± 64.3	10,021^{b,c} ± 43.1	9904^{b,c} ± 86.7
ΣPUFA	45,302^{a,b,c} ± 152	43,519^{b,c,d,e,f} ± 156	45,465^{a,b} ± 42.3	45,148^{a,b,c} ± 132	43,898^{b,c,d,e,f} ± 125	44,668^{a,b,c,d} ± 136
∑SFA/∑UFA	0.82^{c} ± 0.013	0.84^{a,b,c} ± 0.02	0.81^{c} ± 0.01	0.81^{c} ± 0.01	0.83^{c} ± 0.01	0.81^{a,b,c} ± 0.01
ΣMUFA/ΣSFA	0.20^{c,d,e,f,g} ± 0.01	0.22^{a,b,c,d,e} ± 0.01	0.20^{e,f,g} ± 0.01	0.74^{b,c,d,e,f} ± 0.01	0.22^{a,b,c} ± 0.01	0.22^{a,b,c} ± 0.02
18:0/18:1*n*–9c	0.79^{b,c,d,e} ± 0.03	0.76^{d,e,f} ± 0.02	0.78^{c,d,e} ± 0.01	0.74^{f,g,h} ± 0.01	0.76^{e,f,g} ± 0.02	0.69^{i,j} ± 0.01

	L1	L2	L3	L4	L5	L6
14:0	73.9^{a} ± 2.82	71.9^{a} ± 1.88	76.5^{a} ± 2.17	94.5^{a} ± 2.03	86.6^{a} ± 1.60	89.1^{a} ± 1.28
15:0	13.5^{e} ± 0.71	11.5^{e} ± 0.70	12.5^{e} ± 0.71	15.5^{c,d,e} ± 2.10	14.0^{d,e} ± 1.67	13.0^{e} ± 1.83
16:0	35,952^{a,b} ± 157	36,186^{a,b} ± 125	36,357^{a,b} ±142	38,419^{a} ± 121	35,650^{a,b} ± 149	36,058^{a,b} ± 133
16:1	25.4^{a} ± 4.15	28.8^{a} ± 6.20	18.6^{a} ± 11.0	25.8^{a} ± 6.16	26.4^{a} ± 4.14	34.7^{a} ± 1.27
17:0	124^{b} ± 15.4	104^{a} ± 0.03	99.0^{a,b} ± 4.38	107^{a,b} ± 3.83	93.3^{b} ± 0.75	90.7^{b} ± 2.93
18:0	6402^{b,c,d,e} ± 10.5	6503^{c,d,e} ± 18.1	6609^{c,d,e} ± 21.1	6094^{e} ± 35.4	6413^{c,d,e} ± 37.2	6424^{c,d,e} ± 59.8
18:1*n*–9c	8060^{c,d,e} ± 7.26	8812^{g} ± 7.13	9071^{d,e,f} ± 0.41	9131^{c,d,e} ± 37.4	10,106^{a,b} ± 27.8	10,562^{a} ± 66.8
18:2*n*–6c	44,614^{a,b,c,d} ± 126	44,021^{a} ± 4.10	43,353^{a,b,c} ± 13.9	42,070^{d,e,f} ± 127	43,143^{a,b,c,d,e} ± 97.1	42,367^{c,d,e,f} ± 124
20:0	1695^{b,c} ± 34.4	1589^{b,c} ± 76.6	1576^{b,c} ± 49.8	1497^{a} ± 39.3	1632^{b,c} ± 22.5	1606^{b,c} ± 69.5
20:1	213^{b} ± 8.88	202^{b} ± 0.74	215^{b} ± 18.3	217^{b} ± 1.65	243^{b} ± 1.40	239^{b} ± 0.57
18:3*n*–3	1352^{a,b} ± 0.02	1257^{a,b} ± 10.2	1297^{a,b,c} ± 23.8	1113^{d,e} ± 43.3	1028^{e,f} ± 19.4	929^{f} ± 6.95
21:0	18.7^{f} ± 2.79	18.7^{f} ± 4.17	19.2^{f} ± 6.27	31.9^{d,e,f} ± 2.13	32.0^{d,e,f} ± 2.08	24.6^{e,f} ± 4.18
20:2	32.0^{a,b,c} ± 4.01	29.7^{a,b,c} ± 0.65	32.0^{a,b,c} ± 2.67	26.7^{b,c} ± 3.28	32.0^{a,b,c} ± 1.98	32.9^{a,b,c} ± 0.22
22:0	242^{c,d,e} ± 10.2	203^{c,d,e} ± 6.79	212^{d,e} ± 22.0	195^{d,e} ± 5.47	173^{e} ± 6.21	174^{e} ± 6.19
24:0	31.9^{e,f} ± 5.20	28.3^{g} ± 4.43	43.9^{g} ± 5.19	36.6^{g} ± 7.34	35.6^{g} ± 8.89	45.0^{d,e,f} ± 1.92
ΣSFA	44,553^{b} ± 175	44,715^{b} ± 149	45,005^{b} ± 161	46,476^{a} ± 166	44,117^{b} ± 137	44,512^{b} ± 182
ΣMUFA	8299^{c,d,e,f,g} ± 85.7	9043^{h} ± 63.2	9304^{e,f,g,h} ± 46.6	9374^{c,d,e,f} ± 37.2	10,375^{a,b} ± 103	10,916^{a} ± 194
ΣPUFA	45,998^{a,b,c,d} ± 158	45,307^{a} ± 142	44,682^{a,b,c} ± 55.5	43,183^{d,e,f} ± 131	44,170^{a,b,c,d,e,f} ± 108	43,295^{c,d,e,f} ± 117
∑SFA/∑UFA	0.83^{b,c} ± 0.01	0.82^{c} ± 0.01	0.82^{c} ± 0.01	0.88^{a,b,c} ± 0.06	0.81^{c} ± 0.01	0.82^{c} ± 0.02
ΣMUFA/ΣSFA	0.21^{c,d,e,f,g} ± 0.01	0.19^{g} ± 0.01	0.20^{c,d,e,f,g} ± 0.01	0.22^{c,d,e,f,g} ± 0.01	0.23^{a,b} ± 0.01	0.25^{a} ± 0.01
18:0/18:1*n*–9c	0.73^{g,h} ± 0.02	0.79^{b,c,d,e} ± 0.02	0.74^{f,g,h} ± 0.01	0.67^{j,k} ± 0.01	0.63^{k,l} ± 0.02	0.61^{l} ± 0.02

[a–f] Different letters indicate statistically significant differences between different samples for $p \leq 0.05$.

Table 8
Coffee *Arabica* content (%, w/w) in commercially available coffee mixtures prepared in the laboratory, evaluated according to the AM and MLRM models.

Markers	Coffee samples/% Arabica reported on label									
	C1/100	C2/85	C3/80	C4/80	C5/80	C6/80	C7/70	C8/70	C9/70	C10/60
Σ MUFA	78.55 ± 2.21	62.52 ± 0.76	70.61 ± 0.99	75.36 ± 0.14	73.41 ± 3.79	51.11 ± 0.40	58.64 ± 0.00	69.93 ± 1.50	48.60 ± 1.05	82.50 ± 4.03
C18:3n–3	91.14 ± 0.19	82.56 ± 3.14	74.90 ± 2.73	81.52 ± 2.06	75.05 ± 2.28	51.53 ± 1.85	85.67 ± 0.42	83.49 ± 3.50	68.21 ± 1.18	53.11 ± 3.53
C18:0/C18:1n–9c	99.47 ± 4.17	82.20 ± 2.46	86.35 ± 4.83	84.90 ± 1.50	91.44 ± 2.87	53.07 ± 0.18	90.49 ± 0.59	79.90 ± 2.89	71.20 ± 0.28	77.31 ± 2.23
∑MUFA/∑SFA	84.31 ± 2.93	67.72 ± 3.35	87.84 ± 1.30	81.18 ± 1.04	90.19 ± 1.89	53.70 ± 1.69	63.04 ± 0.71	73.70 ± 3.65	53.97 ± 1.45	82.88 ± 1.33
AM	90.69 ± 2.53	76.00 ± 0.85	79.94 ± 1.31	80.59 ± 0.08	82.02 ± 2.63	57.88 ± 0.10	73.57 ± 0.43	75.40 ± 0.31	62.40 ± 0.85	71.16 ± 1.12
Average error % (AM)	−9.31	−10.59	−0.07	0.74	2.52	−27.65	5.10	7.72	−10.86	18.60
MLRM	111.98	114.03	100.11	92.32	96.16	51.37	127.77	102.08	101.14	71.20
Average error % (MLRM)	−11.98	−34.16	−25.14	−15.40	−20.20	35.78	−82.52	−45.82	−44.49	−18.66

Markers	Coffee samples/% Arabica reported on label			Coffee samples/% Arabica added in the blend					
	C11/50	C12/40	C13/30	L1/87	L2/73	L3/65	L4/50	L5/30	L6/20
Σ MUFA	61.60 ± 2.95	47.45 ± 1.02	67.49 ± 2.07	88.54 ± 2.04	70.78 ± 1.57	64.55 ± 3.50	62.86 ± 1.83	38.98 ± 2.46	26.09 ± 2.64
C18:3n–3	59.24 ± 1.81	69.52 ± 2.46	29.86 ± 3.18	87.66 ± 2.89	72.79 ± 1.56	79.10 ± 3.71	50.29 ± 2.77	37.01 ± 3.03	21.50 ± 1.08
C18:0/C18:1n–9c	64.11 ± 1.48	70.05 ± 1.47	47.35 ± 2.87	81.63 ± 2.05	62.23 ± 1.34	59.03 ± 2.17	37.87 ± 0.96	26.59 ± 0.51	17.47 ± 0.62
∑MUFA/∑SFA	61.45 ± 0.44	50.31 ± 3.29	66.74 ± 1.06	92.52 ± 2.33	74.78 ± 4.13	69.78 ± 2.95	75.42 ± 1.89	38.24 ± 2.80	27.07 ± 2.59
AM	59.28 ± 1.52	55.46 ± 0.83	48.29 ± 0.26	87.47 ±0.60	70.71 ± 1.37	67.49 ± 0.14	55.29 ± 1.74	34.17 ± 0.92	22.43 ± 2.22
Average error % (AM)	18.56	38.66	60.96	0.54	−3.13	3.84	10.59	13.89	12.15
MLRM	59.69	102.43	18.03	81.85	74.14	100.30	40.89	28.03	13.16
Average error % (MLRM)	−1.38	−156.07	38.88	5.92	−1.56	−54.30	18.22	6.58	34.18

sample C1, where the declared percentage was 100% and the calculated percentage of *Arabica* from the AM model was of 90.69%. Samples identified as C6, C10, C11, C12 and C13 had an error percentage greater than 11%. It was possible that in mixtures with less than 50% *Arabica*, the chosen markers were affected by greater errors, for the same reasons previously described. This is the case for samples C12 and C13. For the samples labelled C6, C10 and C11, the composition indicated on the label from the producer was different from the real composition. In fact the varietal compositions reported on the label was 80%, 60% and 50% respectively, for samples C6, C10 and C11, while the compositions calculated using the AM mathematical model were 57.88%, 71.16% and 59.28%, respectively. The use of the MRLM model gave values of 51.35%, 71.20% and 59.69%, respectively. It can be concluded that the proposed AM and MRLM models used (Clarysse et al., 2009; Pradelles et al., 2008) can give quickly an indicative answer on the composition of a coffee mixture. This could represent a useful and suitable tool to assess the amounts of *Arabica* and *Robusta* in a coffee blend.

4. Conclusions

Many studies have been aimed to identify possible indicators to discriminate between *Arabica* and *Canephora (Robusta)* coffees varieties in mixtures, but parameters like the roasting degree of the coffee strongly influenced the results, making it difficult to differentiate and quantify the two varieties in a mixture. The aim of this study was to propose and test a model, based on fatty acid composition of *Arabica* and *Robusta* coffee and to identify markers which allowed discrimination between the two varieties in a mixture. In particular, linoleic and α-linolenic acid were more abundant in *Arabica* coffee, while in *Robusta* contained a greater amount of oleic acid was observed.

∑MUFA, 18:3n3, ∑MUFA/∑SFA and 18:0/18:1n9c were the identified markers that better allowed to discriminate the *Arabica* and *Canephora* (*Robusta*) coffee varieties. These parameters selected by the PCA showed a linear relationship with the percentage of *Arabica* contained in the coffee blends. ∑MUFA, *cis*18:3*n*–3, ∑MUFA/∑SFA and 18:0/*cis*18:1*n*–9 values were used in two linear models in order to estimate the content of *Arabica* in coffee commercial blends. The AM mathematical model was more predictive for the experimental data (L1–L6 blends) in the entire range of composition (0–60%, w/w, of *Arabica*). The variability of fatty acid composition in the *Robusta* variety could explain the lower applicability of the MRLM method in blends containing a greater percentage (w/w) of this variety.

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